

The neuropathology of CJD

Definitive diagnosis of a human prion disease requires neuropathological examination of the brain of the affected individual. In the laboratory investigation of human prion diseases a combined, morphological, immunohistochemical, biochemical and molecular genetic approach is desirable. However, many cases of human prion disease can be confirmed on morphological assessment alone, including the vast majority of cases of sporadic and variant CJD. Continued surveillance of the diverse neuropathological features which occur within this group of neurodegenerative disorders has been instrumental in the identification of a widening spectrum of human prion diseases.

Prion diseases of humans and animals are associated with four common histopathological features generally confined to the central nervous system (CNS). These comprise spongiform vacuolation throughout the cerebral grey matter (Figure 1a), reactive proliferation of astrocytes and microglia (Figure 1b), neuronal loss, and in certain subgroups of prion diseases the formation and deposition of amyloid plaques within the brain (Figure 1c). Spongiform change is the most consistent histological abnormality observed in cases of prion disease, reflected in the more traditional term “spongiform encephalopathy” to describe this group of disorders. Spongiform change is characterised by a fine vacuole-like appearance in the neuropil, with vacuoles varying from approximately 2-200µm in diameter (Figure 2). These vacuoles can appear in any layer of the cerebral cortex, where they may become confluent resulting in large irregular cavities within the neuropil. Examination of spongiform change has shown that the majority of vacuoles occur within neuronal processes (mainly neurites) and cell bodies. In addition to the cerebral cortex spongiform change is frequently observed in the basal ganglia and thalamus. Cerebellar involvement is present in most cases, although the severity and distribution of the spongiform change is more variable in this region of the brain.

The four neuropathological features have formed the basis of the histological diagnosis of human prion disease for many years. None of these features in itself is absolutely specific for prion disease, but their occurrence in defined neuroanatomical regions of the brain is of considerable importance in the differential diagnosis of disease. The distribution and extent of these neuropathological changes shows marked case-to-case variation, even within the

same disease subgroup and within the CNS in individual cases. As a result, extensive histological sampling of the post-mortem brain is essential in cases of suspected prion disease. Brain biopsies are performed less frequently in the diagnosis of prion disease and are usually carried out in patients in which a treatable alternative diagnosis is being considered. However, brain biopsy has been shown to be diagnostic in around 95% of patients who were confirmed as having CJD following post-mortem examination.

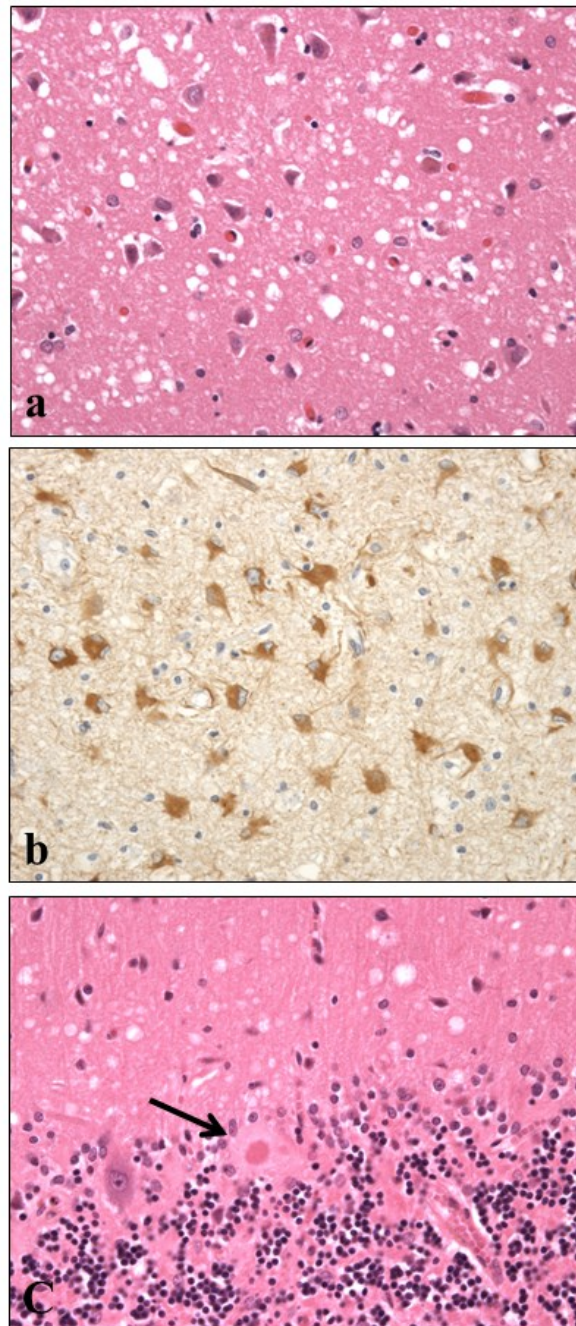


Figure 1: Pathological changes in human prion disease. (a) micro-vacuolar degeneration in the frontal cortex in sporadic CJD (haematoxylin and eosin stain). (b) Astrocytes immunolabelled for glial fibrillary acidic protein in the thalamus of a variant CJD case. (c) A kuru plaque (arrow) within the granular layer of the cerebellum in sporadic CJD.

Most neuropathological studies in human prion disease are performed on paraffin embedded tissues. Tissue blocks from the CNS are decontaminated in 96% formic acid for 1 hour prior to processing into paraffin wax. Sections are then cut for microscopy and stained for routine analysis with haematoxylin and eosin. In addition to conventional histology, the application of immunohistochemical techniques on paraffin embedded tissue sections have demonstrated that prion diseases are normally associated with the deposition of the disease associated protein (PrP^{Sc}) within CNS tissue and in some cases peripheral tissues of affected individuals.

The demonstration of PrP^{Sc} within the brain is a diagnostic feature of all prion diseases. A wide range of antibodies and immunohistochemical protocols are now available for the sensitive detection of PrP in paraffin embedded tissue sections. The majority of these anti-PrP antibodies are unable to distinguish between the normal and disease-associated prion protein. In order to improve the specificity in the detection of PrP^{Sc}, a variety of pre-treatment steps are included in the immunohistochemical detection of PrP. In human prion diseases, variations in the patterns of PrP have been described ranging from fine punctate positivity in a synaptic or perineuronal pattern to a more intense peri-vacuolar pattern of deposition. Although less sensitive in the detection of PrP when compared to Western blot analysis and the more recent *in vitro* models of PrP^{Sc} detection, immunohistochemistry is superior in its ability to demonstrate the cellular and subcellular localization of PrP^{Sc} in the brain. Investigations on the variation and targeting of PrP pathology to particular regions and cell types in the brain is an important diagnostic tool in the differential diagnosis of the sporadic, genetic, and acquired forms of human prion diseases. The major neuropathological features which characterise and distinguish cases of sporadic CJD from variant CJD are summarised below.

Sporadic CJD

As with clinical features, sporadic CJD shows a great diversity in neuropathology features, specifically in the nature, severity and the location of spongiform change, the presence or absence of amyloid plaques and in the pattern of PrP deposition. Sporadic CJD can be sub classified according to the polymorphism at position 129 in the prion protein gene (*PRNP*) – MM, MV or VV, and the isoform of the abnormal prion protein detected on western blot examination of brain homogenate – PrP^{res} type 1 or type 2. The commonest subtype is the MM1/MV1 subtype, followed by VV2, MV2, and VV1. The MM2 subtype exists in 2 forms, cortical and thalamic, identified as MM2C and MM2T. In sporadic CJD there appears to be a

correlation between the diverse clinical and neuropathological features with certain combinations of the *PRNP* codon 129 genotype and PrP^{res} isotype of the individual. The neuropathological features in sCJD as classified according to combinations of *PRNP* codon 129 and PrP^{res} type summarised in Table 1.

Table 1. Predominant neuropathological features observed within the major subtypes of sporadic CJD classified according to *PRNP* codon 129 genotype and PrP^{res} isotype.

Subtype	Frequency(%)	Distribution of vacuolation	Patterns of PrP deposition
MM1	57	Micro-vacuolation within the cerebral cortex, most prominent in the occipital cortex. Confluent vacuoles are observed in around one third of cases	“synaptic” deposition of PrP with perivacuolar deposition around areas of confluent spongiform change
MM2C	7	Large confluent vacuoles	Perivacuolar labelling for PrP in all cortical layers
MM2T (sporadic fatal insomnia)	<1%	Spongiform change may be absent or focal areas.	PrP is detected in lower amounts than in all other subtypes
MV1	6	Micro-vacuolation within the cerebral cortex, most prominent in the occipital cortex. Confluent vacuoles are observed in around one third of cases	“synaptic” deposition of PrP with perivacuolar deposition around areas of confluent spongiform change
MV2	14	Vacuolation within entorhinal cortex, striatum, thalamus and hippocampus	Kuru plaques in the cerebellum with prominent perineuronal labelling in the cortical layers
VV1	2	Extensive micro-vacuolation in the cerebral cortex and striatum	Faint synaptic labelling
VV2	14	Widespread micro-vacuolation vacuolation in the cerebellum, striatum, thalamus and hippocampus	Prominent perineuronal labelling in the cortical layers with numerous plaque-like deposits and some synaptic PrP deposits

Spongiform change in sporadic CJD can vary from focal areas of micro-vacuolation, as described in the MM1 and VV2 subtypes (Figure 2a) to areas of extensive confluent spongiform change, a characteristic feature of the MM2C subtype (Figure 2b). In the most severe cases of sporadic CJD, including the pan-encephalopathic forms there may be status spongiosis accompanied by extensive neuronal loss and collapse of the cerebral cortical cytoarchitecture, leaving an irregular distorted rim of gliotic tissue containing few remaining neurons (Figure 3). Amyloid plaque formation is not a consistent feature in sporadic CJD and is observed only in the MV2 subtype. In these cases, plaques are most frequently observed in the granular and molecular layer of the cerebellum where they are characterised by a hyaline eosinophilic core and a paler halo. Like the plaques observed in cases of Kuru, these plaques often show a peripheral margin of radiating fibrils (Figure 4a).

The presence of amyloid plaques is most easily visualised following immunohistochemical staining with an anti-PrP antibody (Figure 4b). Three main patterns of PrP deposition are described in cases of sporadic CJD; plaque type, diffuse synaptic or granular and patchy/perivacuolar types. Examples of these different PrP labelling patterns are shown in Figure 5.

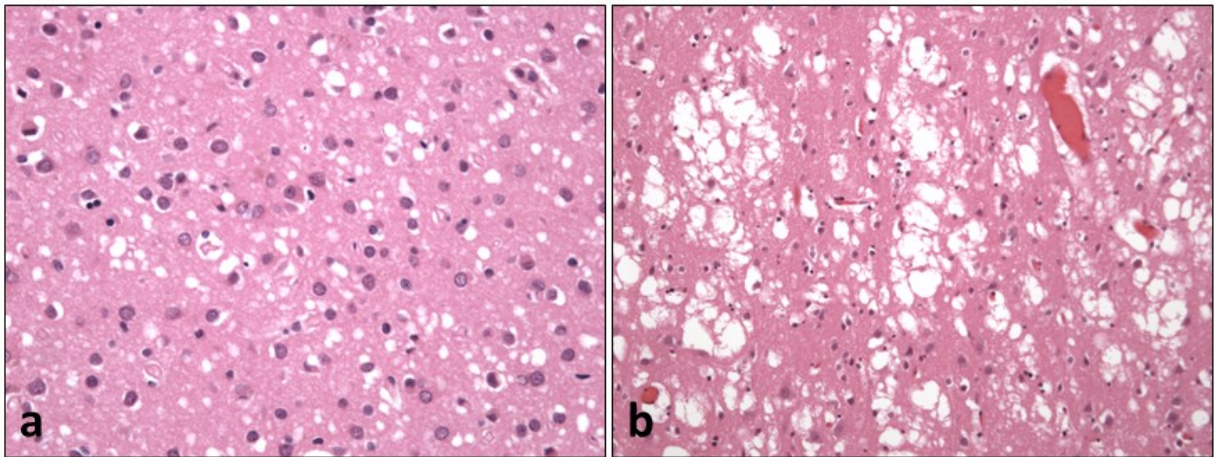


Figure 2: Spongiform change in sporadic CJD. (a) micro-vacuolation characterised by multiple small rounded vacuoles within the neuropil of the frontal cortex in the MM1 subtype. (b) The MM2 subtype shows widespread confluent spongiform change in the cerebral cortex.

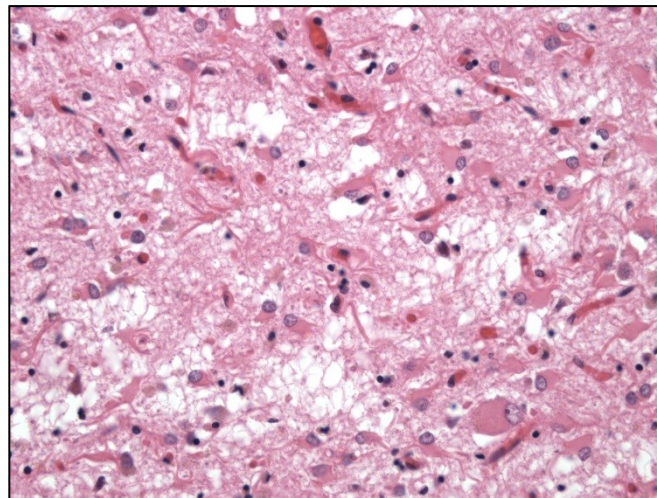


Figure 3: Severe spongiform vacuolation in sporadic CJD results in status spongiosus with extensive neuronal loss, widespread astrogliosis and the resulting collapse of the cerebral cytoarchitecture.

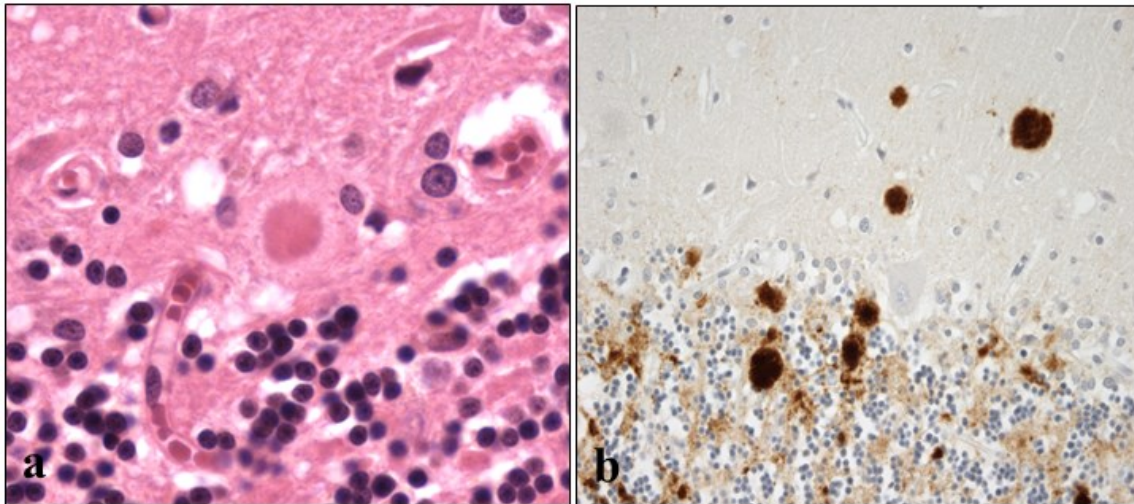


Figure 4: Amyloid plaque deposition in the MV2 subtype of sporadic CJD. (a) kuru plaque in the cerebellum showing rounded fibrillary structure with a dense core and pale periphery. (b) Numerous kuru-like plaques within the granular and molecular layer of the cerebellum following immunohistochemistry for PrP (KG9 antibody).

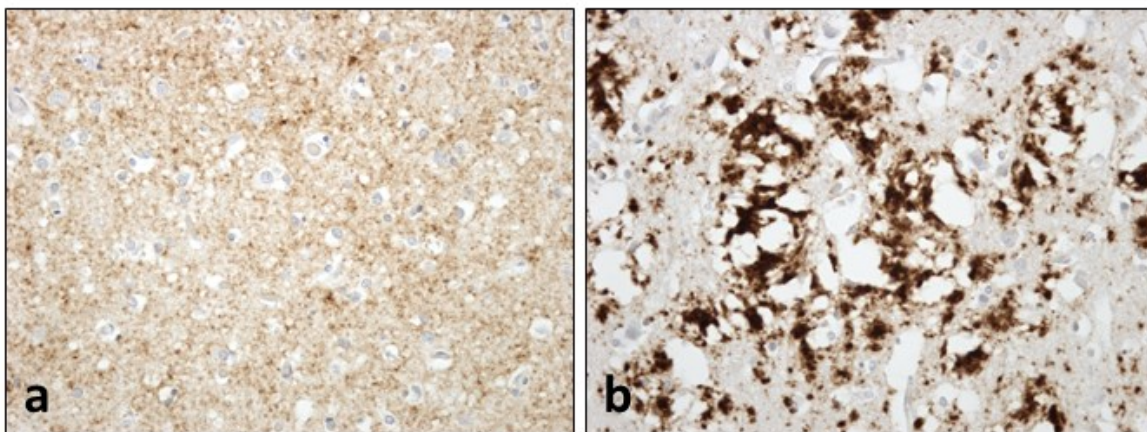


Figure 5: Patterns of PrP deposition in sporadic CJD. (a) Widespread deposition of PrP in a predominantly synaptic pattern in the MM1 subtype. (b) Intense immunoreactivity for PrP around confluent spongiform change in the MM2 subtype. KG9 anti-PrP antibody.

Variant CJD

In contrast to the marked heterogeneity in the neuropathological features of sporadic CJD, variant CJD is characterised by a highly stereotyped pathology. The most striking feature is the deposition of ‘florid’ plaques most prominently found within the occipital cortex and cerebellum. Florid plaques have the characteristic appearance of classic kuru-type plaques surrounded by a corona or halo of spongiform change (Figure 6a). Spongiform change is most severe in the thalamus but is also a prominent feature throughout the cerebral cortex and cerebellum (Figure 6b). Variant CJD shows a distinct pattern of PrP positivity. In addition to the florid plaques, a large number of smaller plaque-like deposits are observed in cerebral

cortex accompanied by widespread peri-cellular accumulations (Figure 7a). A unique neuropathological feature in variant CJD is the punctate deposition of PrP in a linear formation in the basal ganglia (Figure 7b). Variant CJD is unique to other human prion diseases in that PrP^{Sc} is readily detected out with the CNS. Immunohistochemistry for PrP has demonstrated deposition of PrP in the lymphoreticular system within the lymphoid follicles in association with follicular dendritic cells (FDCs) (Figure 8). PrP deposition in the lymphoreticular system occurs prior to the onset of clinical systems. This is reflected in the inclusion of a positive tonsil biopsy as one of the diagnostic criteria for variant CJD. All patients confirmed with variant CJD after neuropathological examination have been methionine homozygotes (MM) at codon 129 in the *PRNP* gene. Western blot examination of brain homogenate and lymphoid tissue homogenates shows an isoform of disease-associated prion protein that is distinct from sporadic CJD, classified as the type 2B isoform.

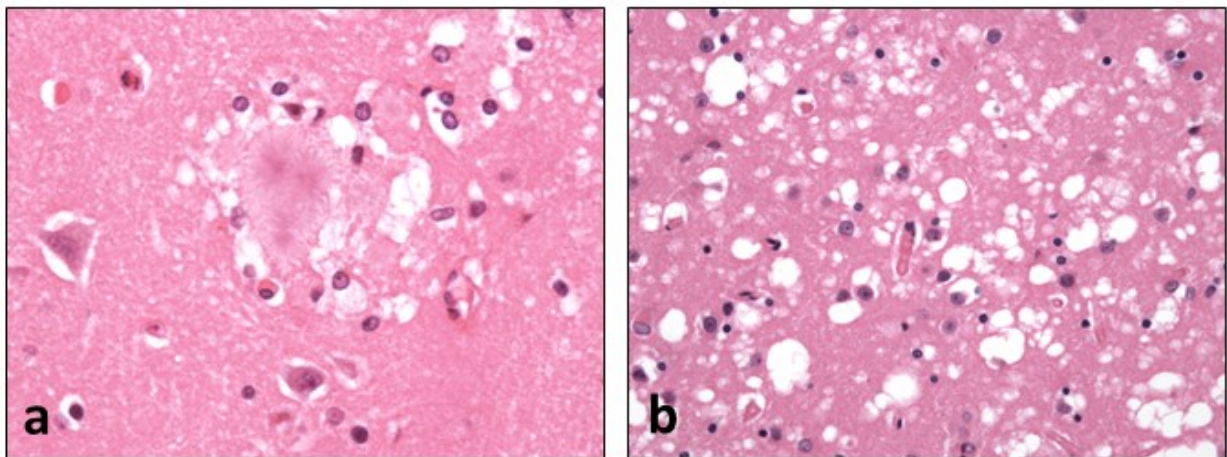


Figure 6: (a) A cluster of large florid plaques within the cerebral cortex in variant CJD. (b) Widespread vacuolation within the caudate nucleus of the basal ganglia

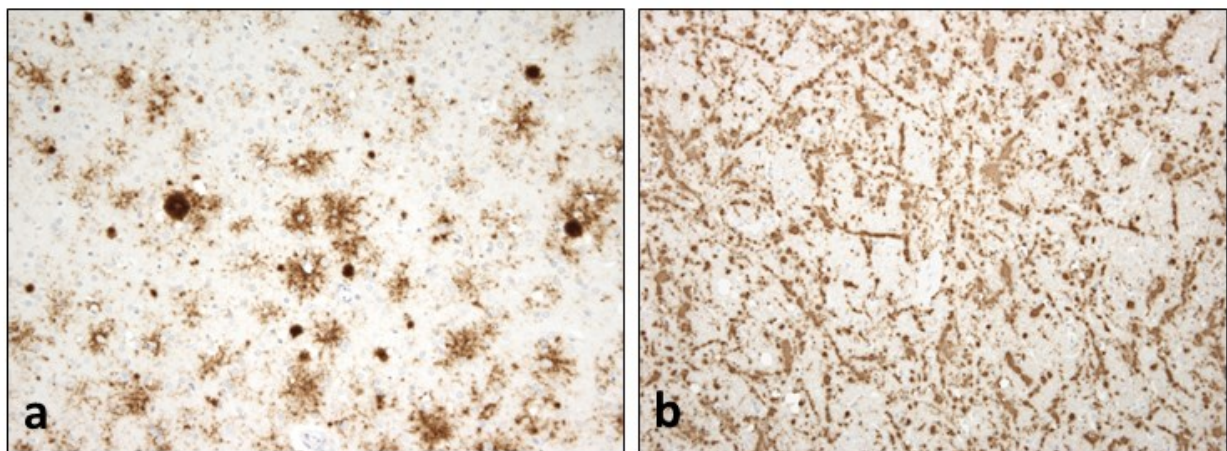


Figure 7: Prion protein immunohistochemistry in variant CJD. (a) Intense immunolabelling of plaques and cluster plaques in the frontal cortex. In addition, peri-cellular deposits of PrP are a consistent feature in the cerebral cortex. (b) The caudate nucleus of the thalamus showing a distinctive linear pattern of PrP deposition.

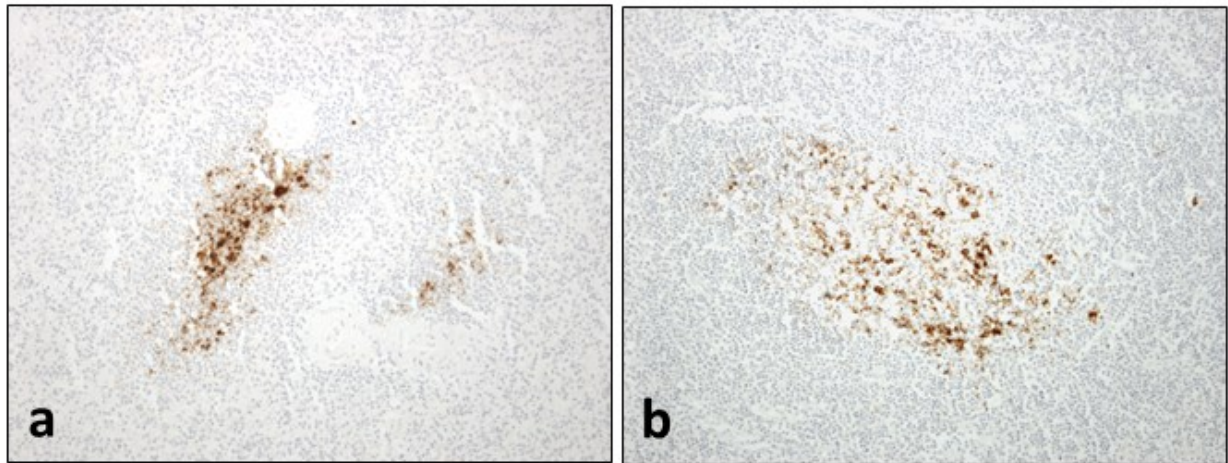


Figure 8: Prion protein immunohistochemistry within the (a) spleen and (b) tonsil in variant CJD. KG9 ant-PrP antibody

FURTHER READING

Ironside, J.W., Ritchie, D.L., and Head, M.W. (2005). Phenotypic variability in human prion disease. *Neuropathol Appl Neurobiol* 31, 565-579.

Ironside, J.W. (2008) *Greenfield's Neuropathology*. pp. 573-574. Wiley online library

Parchi, P., Giese, A., Capellari, S., Brown, P., Schulz-Schaeffer, W., Windl, O., Zerr, I., Budka, H., Kopp, N., Piccardo, P., Poser, S., Rojiani, A., Streichemberger, N., Julien, J., Vital, C., Ghetti, B., Gambetti, P., and Kretzschmar, H. (1999). Classification of sporadic Creutzfeldt-Jakob disease based on molecular and phenotypic analysis of 300 subjects. *Ann. Neurol* 46, 224-233.